Supplementary material for:

Aggregation behavior of the amyloid model peptide NACore

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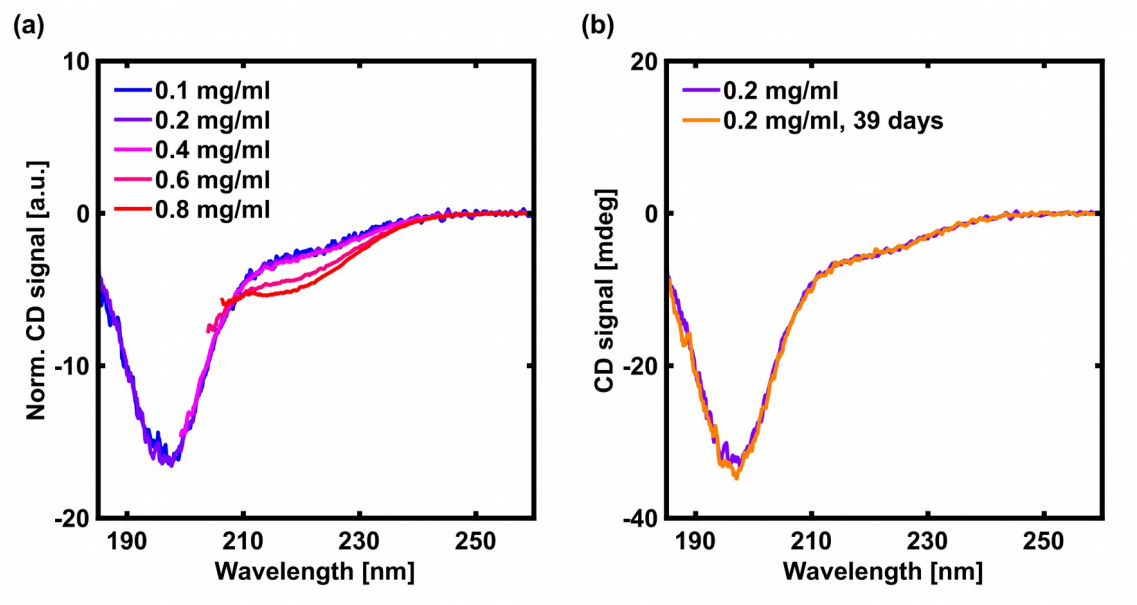

Fig. S1. (a) Estimation of peptide solubility at high pH (2 mM NaOH, pH 11.3). The CD spectra of different concentrations of lyophilized peptide powder is shown. The CD signal has been normalized with the concentration, and is only shown for HT voltage below 700 V (that is why the spectra for the highest concentrations are truncated). Up to 0.4 mg/ml the normalized spectra are almost identical. At concentrations of 0.6 mg/ml and 0.8 mg/ml a partial change in the spectra is apparent, suggesting that the peptide is no longer fully disordered. (b) The unnormalized CD spectrum of the 0.2 mg/ml sample in a, together with another sample of the same type that had been left standing in the lab at room temperature for 39 days. The two CD spectra almost perfectly overlap, indicating that the NACore peptide does not fibrillate in the high pH solution condition used as the initial state for the fibrillation experiments.

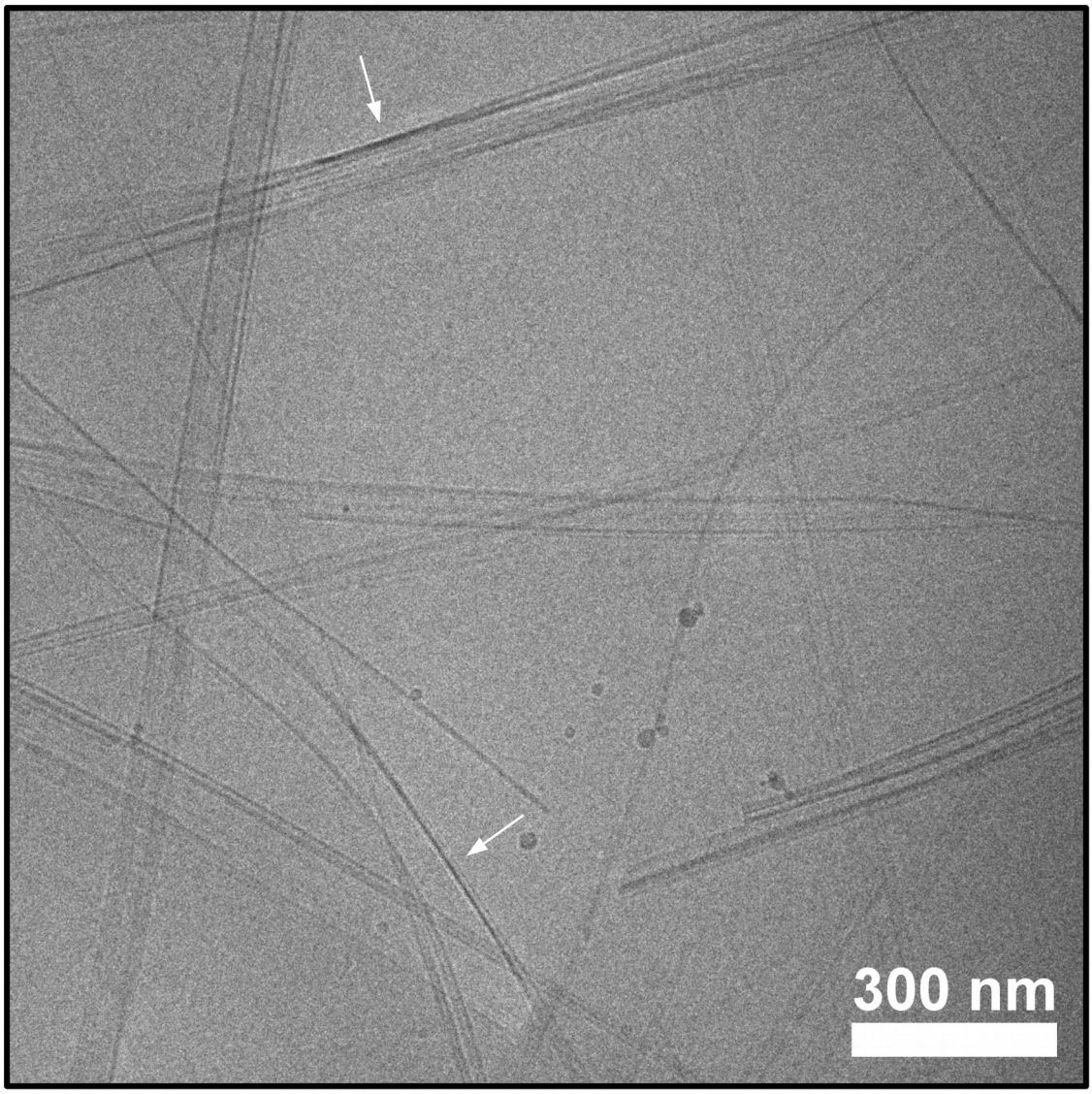

Fig. S2. Cryo-TEM image (not zero-loss) of what appears to be occasional twists in the peptide fibrils (white arrows). The sample was the same one as in Fig. 3b.

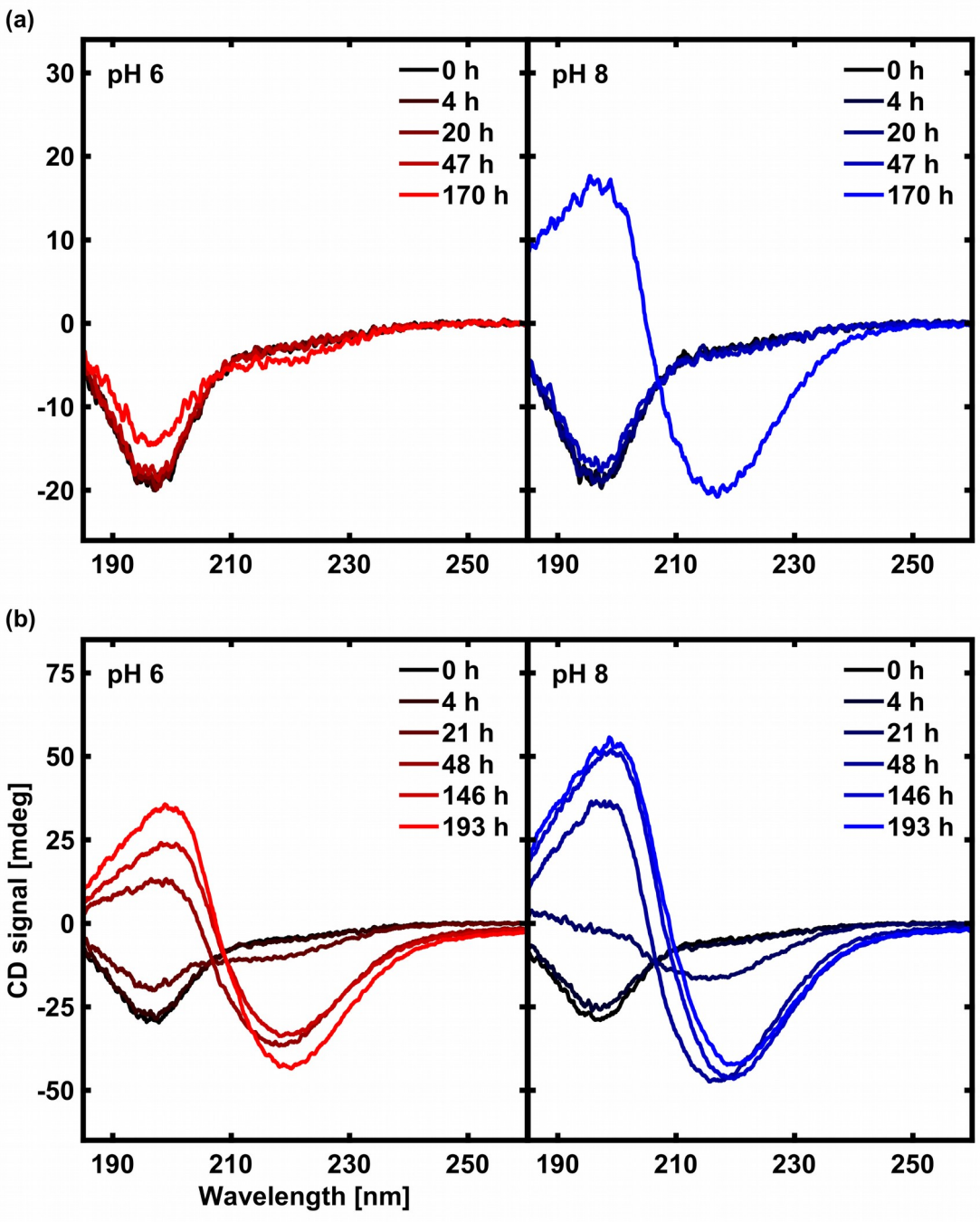

Fig. S3. (a) Fibrillation kinetics under the two different solution conditions (pH 6 and pH 8) and at a final nominal lyophilized peptide concentration of 0.1 mg/ml for another batch of synthetic NACore peptide than the one used for the kinetics experiments in Fig. 6 of the main paper. (b) Same as in a, but with a 1.5 times higher peptide concentration (0.15 mg/ml). In both cases the relative difference in behavior between the pH 6 and pH 8 conditions remains in that the pH 6 condition leads to slightly slower rather than faster fibrillation. However, when compared to the kinetics of the experiment presented in Fig. 6 of the main paper the kinetics is slower here, primarily due to a long lag phase, which we did not observe with the other batch. The duration of the lag phase appears to be concentration dependent, because it is substantially shorter in b than in a.

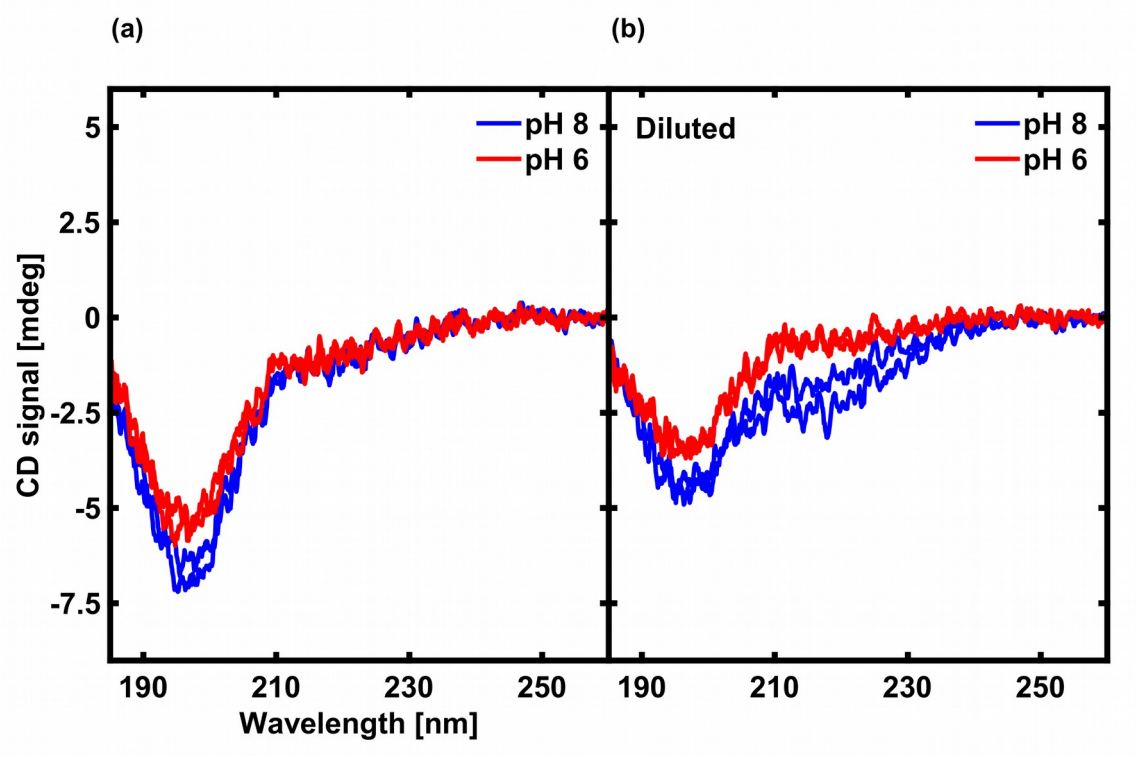

Fig. S4. Investigation of the amount of free peptide remaining at the end of the fibrillation process (same peptide batch as for Fig. S3). See section S1 for a detailed description of this experiment.

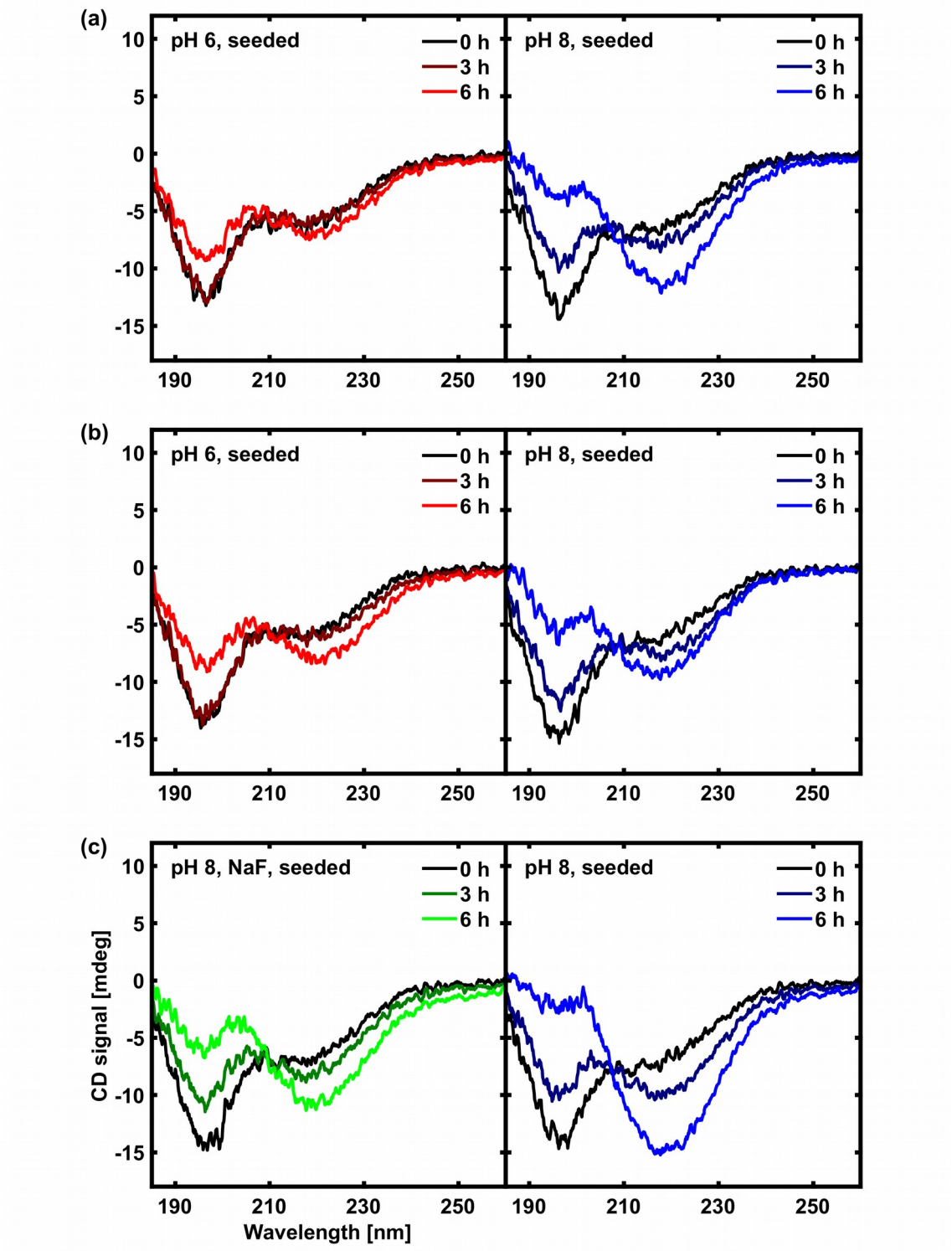

Fig. S5. (a) Seeded fibrillation kinetics using peptide from the same batch as for the experiments in Fig. S3. (b) Replicate of a. (c) Comparison between two different ionic strengths at the same pH. See section S2 for a detailed description of the experiments.

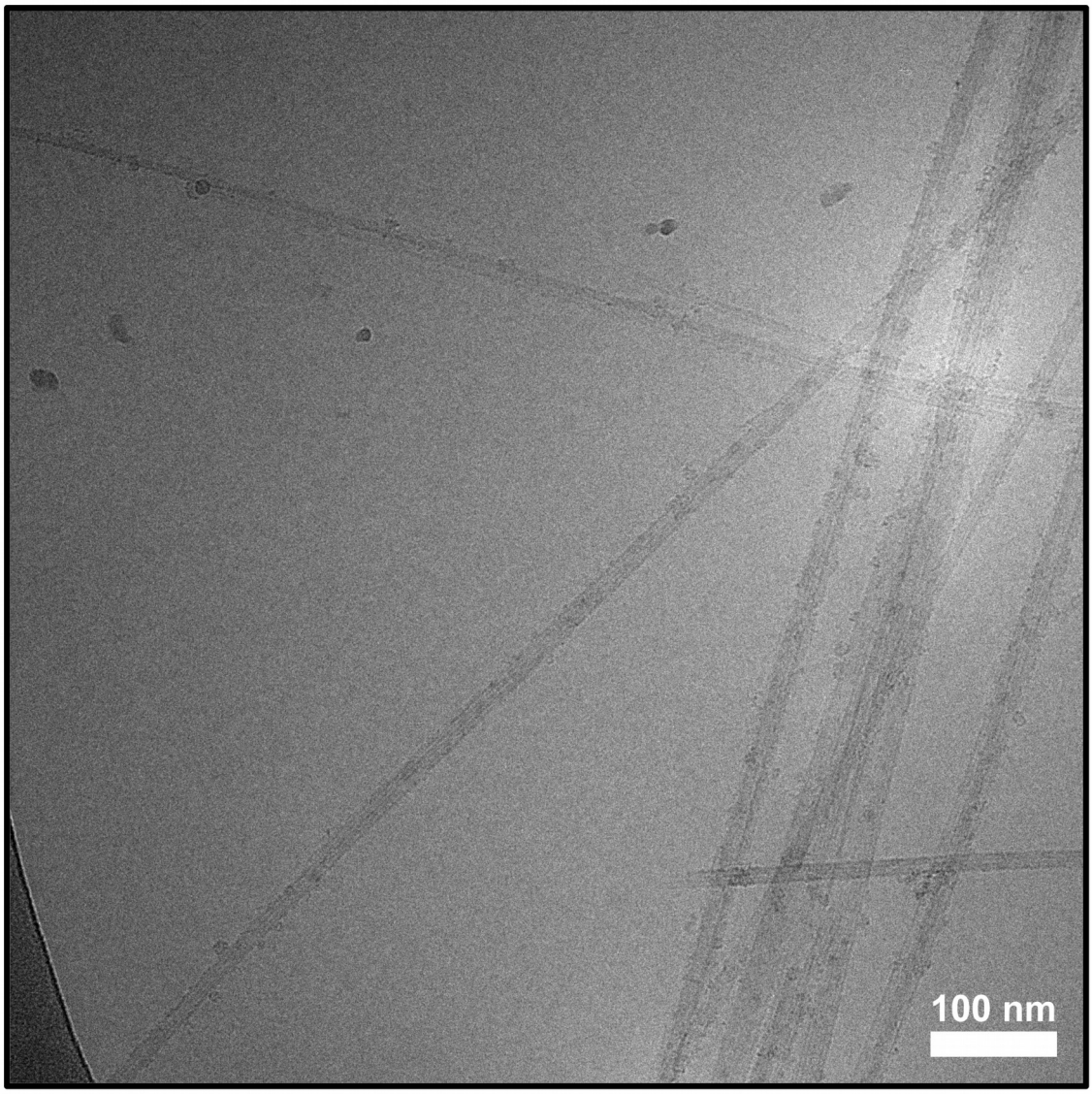

Fig. S6. Cryo-TEM image (zero-loss with a 10 eV slit) of a sample prepared in the same way as the seeded pH 6 samples in Fig. S5. The sample was plunge frozen at the 3 h time point. That is, at the time when preformed fibrils had been added, but no change from the initial CD spectrum had been observed. Several fibrils can be observed in the image, and these are covered with what appears to be amorphous structures along the sides. This is a fully zoomed out version of the image shown in Fig. 5a of the main paper.

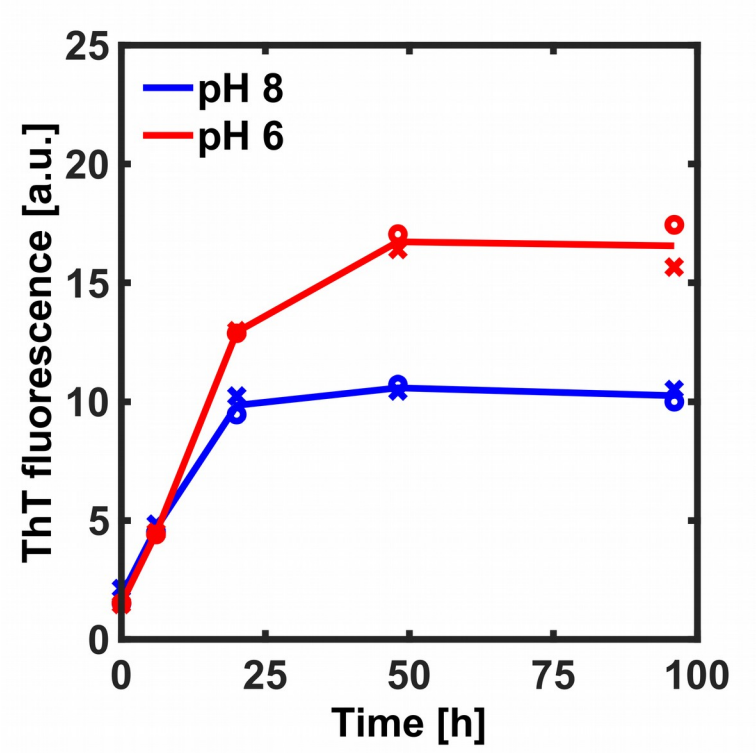

Fig. S7. ThT fluorescence from peptide samples after lowering the pH to either about 6 or 8 as a function of time. The circles and crosses show data points for two separate sets of samples. This is from the same measurements as in Fig. 6d, except that the fluorescence is not normalized.

S1. Supplementary solubility experiment (Fig. S4)

Samples were prepared in the same way as described in the main paper, except that the final lyophilized peptide concentration was 0.2 mg/ml instead of 0.1 mg/ml. After one week of incubation, parts of the samples were centrifuged for 60 min at 16 000 rcf. Circular dichroism (CD) spectra of the supernatants were then measured, and the resulting spectra for duplicate samples at each pH condition are shown in Fig. S4a. Based on the CD spectra most of the peptide was in the pellet for both pH conditions, and the peptide that remained in the supernatant had a disordered structure. The amplitudes of the signal correspond to peptide concentrations of about 0.04 mg/ml (0.04 mM) and 0.03 mg/ml (0.03 mM) for the pH 8 and pH 6 conditions, respectively. To test whether these concentrations represented the true equilibrium peptide solubilities, we had also diluted parts of each sample by a factor of two in the corresponding buffers before the centrifugation step. The diluted samples were then left to equilibrate for 3 h before they were centrifuged, followed by measurement of the CD spectra of the supernatants (Fig. S4b). In the case of true equilibrium we would expect the spectra to be the same as for the undiluted samples, due to dissolution of larger aggregates in the diluted samples. However, the spectra for the diluted samples had lower amplitudes, suggesting that the previous concentrations did not represent the true equilibrium solubilities, or that dissolution of the large aggregates in the samples would require substantially more time than 3 h. Interestingly, the pH 8 samples appeared to have a partial β-sheet contribution to their spectra. This could be due to the presence of small β-sheet containing aggregates occurring as intermediate steps during dissolution of larger aggregates, such as pieces of fibrils too small to be pelleted during the centrifugation step.

S2. Supplementary seeded aggregation experiments (Fig. S5)

Samples were prepared in the same way as the ones in Fig. S3a, except that the samples were mixed with a suspension of preformed fibrils in a ratio of 20 to 1 immediately after lowering the pH. The suspension of preformed fibrils had been prepared using the pH 8 solution condition with 3 days of incubation, and had a final peptide concentration of 0.2 mg/ml. The addition of preformed fibrils resulted in a strong reduction of the lag phase, showing that the system was sensitive to seeding. There is an interesting difference in the behavior between the two pH conditions in how the samples reacted to the presence of preformed fibrils. This is particularly prominent 3 h after the start of the reaction. At this time the pH 6 sample showed essentially no change in its CD spectrum from the 0 h spectrum, whereas the pH 8 sample already showed a clear change in its spectrum. This difference in behavior, together with the associated cryo-TEM observations in Fig. 5a and Fig. S6 is very consistent with the idea that the NACore peptide initially forms amorphous aggregates in the pH 6 condition, and that this is related to a somewhat slower overall fibrillation process than in the pH 8 condition. Fig. S4b shows a separately prepared replicate of the experiment in a. The same difference between the two solution conditions is observed. In the experiment presented in Fig. S5c the pH 6 condition had been replaced with a pH 8 sample containing 150 mM added NaF to test the influence of ionic strength. In this case there is no clear difference between the two conditions (at least after 3 h), suggesting that pH is more important than ionic strength for the aggregation behavior. However, the specific ions used in the buffer could also be of importance. In this case NaF was chosen because it does not absorb the far UV light used in the CD measurements.